**Amendments to the Specification:**

Please replace the paragraph bridging pages 3 and 4, with the following amended paragraph:

The thymus is arguably the major organ in the immune system because it is the primary site of production of T lymphocytes. Its role is to attract appropriate bone marrow-derived precursor cells from the blood, and induce their commitment to the T cell lineage including the gene rearrangements necessary for the production of the T cell receptor for antigen (TCR). Associated with this is a remarkable degree of cell division to expand the number of T cells and hence ~~increase~~ increases the likelihood that every foreign antigen will be recognized and eliminated. A unique feature of T cell recognition of antigen, however, is that unlike B cells, the TCR only recognizes peptide fragments physically associated with MHC molecules; normally this is self MHC and this ability is selected for in the thymus. This process is called positive selection and is an exclusive feature of cortical epithelial cells. If the TCR fails to bind to the self MHC/peptide complexes, the T cell dies by "neglect" – it needs some degree of signalling through the TCR for its continued maturation.

Please replace the paragraph at page 8, lines 2-8, with the following amended paragraph:

The present inventors have demonstrated that thymic atrophy (~~aged-induced~~ age-induced, or as a consequence of conditions such as chemotherapy or radiotherapy) can be profoundly reversed by inhibition of sex steroid production, with virtually complete restoration of thymic structure and function. The present inventors have also found that the basis for this thymus regeneration is in part due to the initial expansion of precursor ~~cells which~~ cells, which are derived both intrathymically and via the blood

stream. This finding suggests that is possible to seed the thymus with exogenous haemopoietic stem cells ~~(HSC) which~~ (HSC), which have been injected into the subject.

Please replace the paragraph at page 8, lines 9-13, with the following amended paragraph:

The ability to seed the thymus with genetically modified or exogenous HSC by disrupting sex ~~steroid signalling~~ steroid-signaling to the thymus, means that gene therapy in the HSC may be used more efficiently to treat T cell (and myeloid cells which develop in the thymus) disorders. HSC stem cell therapy has met with little or no success to date because the thymus is dormant and incapable of taking up many if any HSC, with T cell production less than 1% of normal levels.

Please replace the paragraph at page 8, lines 21-25, with the following amended paragraph:

These methods are based on disrupting sex ~~steroid mediated~~ steroid-mediated signaling to the thymus in the subject. In a further embodiment, the subject is post-pubertal. In one embodiment, castration is used to disrupt the sex ~~steroid mediated~~ steroid-mediated signaling. In one embodiment, chemical castration is used. In another embodiment, surgical castration is used. Castration reverses the state of the thymus to its pre-pubertal state, thereby reactivating it.

Please replace the paragraph bridging pages 8 and 9, with the following amended paragraph:

In certain embodiments, inhibition of sex steroid production is achieved by either castration or administration of a sex steroid ~~analogue(s)~~ analogs. Non-limiting sex steroid ~~analogues~~ analogs include eulexin, goserelin, leuprolide, dioxalan derivatives, ~~such as~~ triptorelin, meterelin, buserelin, histrelin, nafarelin, lutrelin, leuprorelin, and luteinizing hormone-releasing hormone ~~analogues~~ analogs. In some embodiments, the sex steroid ~~analogue~~ analog is an ~~analogue~~ analog of luteinizing hormone-releasing hormone. In certain embodiments, the luteinizing hormone-releasing hormone ~~analogue~~ analog is deslorelin.

Please replace the paragraph at page 9, lines 3-6, with the following amended paragraph:

In a particular embodiment sex ~~steroid-mediated~~ steroid-mediated signaling to the thymus is blocked by the administration of GnRH, GnRH analogs, or of agonists or antagonists of LHRH, anti-estrogen antibodies, anti-androgen antibodies, passive (antibody) or active (antigen) anti-LHRH vaccinations, or combinations thereof ("blockers").

Please replace the paragraph at page 9, lines 19-25, with the following amended paragraph:

In cases where the subject is infected with HIV, the HSC may be genetically modified such that they and their progeny, in particular T cells, macrophages and

dendritic cells, are resistant to infection ~~and / or~~ and/or destruction with the HIV virus. The genetic modification may involve introduction into the HSC of one or more nucleic acid molecules which prevent viral replication, assembly and/or infection. The nucleic acid molecule may be a gene which ~~encloded~~ encodes an antiviral protein, an antisense construct, a ribozyme, a dsRNA and a catalytic nucleic acid molecule.

Please replace the paragraph on page 10, lines 14-22, with the following amended paragraph:

The method of the present invention is particularly relevant for treatment of AIDS, where the treatment preferably involves reduction of viral load, reactivation of thymic function through inhibition of sex steroids and transfer into the patients of HSC (autologous or from a second party donor) which have been genetically modified such that all progeny (especially T cells, DC) are resistant to further HIV infection. This means that not only will the patient be depleted of HIV virus and no longer susceptible to general infections because the T cells have returned to normal levels, but the new T cells being resistant to HIV will be able to remove any remnant viral infected cells. In principle a similar strategy could be applied to gene therapy in HSC for any T cell defect or any viral infection which targets T cells.

Please replace the paragraph bridging pages 26-27, with the following amended paragraph:

To generate new T lymphocytes, the thymus requires precursor cells; these can be derived from within the organ itself for a short time, but by 3-4 weeks, such cells are depleted and new hematopoietic stem cells (HSC) must be taken in (under normal

circumstances this would be from the bone marrow via the blood). However, even in a normal functional young thymus, the intake of such cells is very low (sufficient to maintain T cell production at homeostatically regulated levels. Indeed the entry of cells into the thymus is extremely limited and effectively restricted to HSC (or at least prothymocytes which already have a preferential development along the T cell lineage). In the case of the thymus undergoing rejuvenation due to a loss of sex steroid inhibition, this organ has been demonstrated to now be very receptive to new precursor cells circulating in the blood, such that the new T cells which develop from both intrathymic and external precursors. By increasing the level of the blood precursor cells, the T cells derived from them will progressively dominate the T cell pool. This means that any gene introduced into the precursors (HSC) will be passed onto all progeny T cells and eventually be present in virtually all of the T cell pool. The level of dominance of these cells over those derived from endogenous host HSC can be easily increased to very high levels by simply increasing the number of transferred exogenous HSC.

Please replace the paragraph on page 27, lines 11-16, with the following amended paragraph:

The recipient's thymus may be reactivated by disruption of sex ~~steroid mediated signalling~~ steroid-mediated signaling to the thymus. This disruption reverses the hormonal status of the recipient. In certain embodiments, the recipient is post-pubertal. According to the methods of the invention, the hormonal status of the recipient is reversed such that the hormones of the recipient approach pre-pubertal levels. By lowering the level of sex steroid hormones in the recipient, the signalling of these hormones to the thymus is lowered, thereby allowing the thymus to be reactivated.

Please replace the paragraph on page 27, lines 17-23, with the following amended paragraph:

A non-limiting method for creating disruption of sex ~~steroid-mediated signalling~~ steroid-mediated signaling to the thymus is through castration. Methods for castration include, but are not limited to, chemical castration and surgical castration. During or after the castration step, hematopoietic stem or progenitor cells, or epithelial stem cells, from the donor are transplanted into the recipient. These cells are accepted by the thymus as belonging to the recipient and become part of the production of new T cells and DC by the thymus. The resulting population of T cells recognize both the recipient and donor as self, thereby creating tolerance for a graft from the donor.

Please replace the paragraph at page 29, lines 10-23, with the following amended paragraph:

_____Administration may be by any method which delivers the sex ~~steroid-ablating~~ steroid-ablating agent into the body. Thus, the sex ~~steroid-ablating~~ steroid-ablating agent ~~maybe~~ may be administered, in accordance with the invention, by any route including, without limitation, intravenous, subdermal, subcutaneous, intramuscular, topical, and oral routes of administration. One non-limiting example of administration of a sex ~~steroid-ablating~~ steroid-ablating agent is a subcutaneous/intradermal injection of a "slow-release" depot of GnRH agonist (*e.g.*, one, three, or four month Lupron® injections) or a subcutaneous/intradermal injection of a "slow-release" GnRH-containing implant (*e.g.*, one or three month Zoladex®, *e.g.*, 3.6 mg or 10.8 mg implant). These could also be given ~~intramuscular~~ intramuscularly (i.m.), intravenously (i.v.) or orally, depending on the appropriate formulation. Another example is by

subcutaneous injection of a "depot" or "impregnated implant" containing, for example, about 30 mg of Lupron® (*e.g.*, Lupron Depot®, (leuprolide acetate for depot suspension) TAP ~~Pharmaceuticals~~ Pharmaceutical Products, Inc., Lake Forest, IL). A 30 mg Lupron® injection is sufficient for four months of sex steroid ablation to allow the thymus to rejuvenate and export new naïve T cells into the blood stream.

Please replace the paragraph bridging pages 29 and 30, with the following amended paragraph:

In some embodiments, sex steroid ablation or inhibition of sex ~~steroid-signaling~~ steroid-signaling is accomplished by administering an anti-androgen such as an androgen blocker (*e.g.*, bicalutamide, trade names Cosudex® or Casodex®, AstraZeneca, ~~Aukland~~ Auckland, NZ), either alone or in combination with an LHRH analog or any other method of castration. Sex steroid ablation or interruption of sex ~~steroid-signaling~~ steroid-signaling may also be accomplished by administering cyproterone acetate (trade name, Androcor®, ~~Shering~~ Schering AG, Germany; *e.g.*, 10-1000 mg, 100 mg bd or tds, or 300 mg IM weekly, a 17-hydroxyprogesterone acetate, which acts as a progestin, either alone or in combination with an LHRH analog or any other method of castration. Alternatively, other anti-androgens may be used (*e.g.*, antifungal agents of the imidazole class, such as ~~liarozole(Liazol® *e.g.*, 150 mg/day, an aromatase inhibitor)~~ liarozole (Liazol®, *e.g.*, 150 mg/day, an aromatase inhibitor) and ketoconazole, bicalutamide (trade name Cosudex® or Casodex®, 5-500 mg, *e.g.*, 50 mg po QID), flutamide (trade names Euflex® and Eulexin®, ~~Shering~~ Schering Plough Corp, N.J.; 50-500 mg *e.g.*, 250 or 750 po QID), megestrol acetate (Megace®) *e.g.*, 480-840 mg/day or nilutamide (trade names Anandron®, and Nilandron®, Roussel, France *e.g.*, orally, 150-300 mg/day)). Antiandrogens are often important in therapy, since they are

commonly utilized to address flare by GnRH analogs. Some antiandrogens act by inhibiting androgen receptor translocation, which interrupts negative feedback resulting in increased testosterone levels and minimal loss of libido/potency. Another class of anti-androgens useful in the present invention are the selective androgen receptor modulators (SARMS) (*e.g.*, quinoline derivatives, bicalutamide (trade name Cosudex® or Casodex®, ICI Pharmaceuticals, England *e.g.*, orally, 50 mg/day), and flutamide (trade name Eulexin®, *e.g.*, orally, 250 mg/day)). Other well known anti-androgens include 5 alpha reductase inhibitors (*e.g.*, ~~dutasteride,(e.g., 0.5 mg/day)~~ dutasteride, (*e.g.*, 0.5 mg/day) which inhibits both 5 alpha reductase isoenzymes and results in greater and more rapid DHT suppression; finasteride (trade name Proscar®; ~~0.5-500mg, e.g.,~~ 0.5-500 mg, *e.g.*, 5 mg po daily), which inhibits ~~5alpha~~ 5 alpha reductase 2 and consequent DHT production, but has little or no effect on testosterone or LH ~~levels);~~ levels).

Please replace the paragraph bridging pages 30 and 31, with the following amended paragraph:

In other embodiments, sex steroid ablation or inhibition of sex ~~steroid-signaling~~ steroid-signaling is accomplished by administering anti-estrogens either alone or in combination with an LHRH analog or any other method of castration. Some anti-estrogens (*e.g.*, anastrozole (trade name Arimidex®), and fulvestrant (trade name Faslodex® ) act by binding the estrogen receptor (ER) with high affinity similar to estradiol and consequently inhibiting estrogen from binding. Faslodex® binding also triggers conformational change to the receptor and down-regulation of estrogen receptors, without significant change in FSH or LH levels. Other non-limiting examples of anti-estrogens are tamoxifen (trade name Nolvadex®); Clomiphene ~~(trade name~~

~~Clomid®)e.g.,50-250mg/day~~ (trade name Clomid®) *e.g.*, 50-250 mg/day, a non-steroidal ER ligand with mixed agonist/antagonist properties, which stimulates release of gonadotrophins; Fulvestrant (trade name Faslodex®; ~~10-1000mg~~ 10-1000 mg, *e.g.*, ~~250mg~~ 250 mg IM monthly); diethylstilbestrol ((DES), trade name Stilphostrol®) ~~e.g.,1-3mg/day~~ *e.g.*, 1-3 mg/day, which shows estrogenic activity similar to, but greater than, that of estrone, and is therefore considered an estrogen agonist, but binds both androgen and estrogen receptors to induce feedback inhibition on FSH and LH production by the pituitary, diethylstilbestrol diphosphate ~~e.g.,50 to 200 mg/day~~ *e.g.*, 50 to 200 mg/day; as well as ~~danazol,, droloxifene~~ danazol, droloxifene, and iodoxyfene, which each act as antagonists. Another class of anti-estrogens which may be used either alone or in combination with other methods of castration, are the selective estrogen receptor modulators (SERMS) (*e.g.*, toremifene (trade name Fareston®, ~~5-1000mg~~ 5-1000 mg, *e.g.*, ~~60mg~~ 60 mg po QID), raloxofene (trade name Evista®), and tamoxifen (trade name Nolvadex®, ~~1-1000mg~~ 1-1000 mg, *e.g.*, ~~20mg~~ 20 mg po bd), which behaves as an agonist at estrogen receptors in bone and the cardiovascular system, and as an antagonist at estrogen receptors in the mammary gland). Estrogen receptor downregulators (ERDs) (*e.g.*, tamoxifen (trade name, Nolvadex®)) may also be used in the present invention.

Please replace the paragraph bridging pages 31 and 32, with the following amended paragraph:

Other non-limiting examples of methods of inhibiting sex ~~steroid-signalling~~ steroid-signaling which may be used either alone or in combination with other methods of castration, include aromatase inhibitors and other adrenal gland blockers (*e.g.*, Aminoglutethimide, formestane, vorazole, exemestane, anastrozole (trade name

Arimidex®, ~~0.1-100mg~~ 0.1-100 mg, *e.g.*, 1 mg po QID), which lowers estradiol and increases LH and testosterone), letrozole (trade name Femara®, 0.2-500 mg, *e.g.*, ~~2.5mg~~ 2.5 mg po QID), and exemestane ~~(trade name Aromasin®)1-2000mg, *e.g.*, 25mg/day)~~ (trade name Aromasin®, 1-2000 mg, *e.g.*, 25 mg/day); aldosterone antagonists (*e.g.*, spironolactone (trade name, Aldactone®) *e.g.*, ~~100 to 400mg/day~~ 100 to 400 mg/day), which blocks the androgen cytochrome P-450 receptor;) and eplerenone, a selective aldosterone-receptor antagonist) antiprogestogens (*e.g.*, medroxypregesterone acetate, ~~*e.g.* 5mg/day~~ *e.g.*, 5 mg/day, which inhibits testosterone syntheses and LH synthesis); and progestins and anti-progestins such as the selective progesterone response modulators (SPRM) (*e.g.*, megestrol acetate ~~*e.g.*,160mg/day~~ *e.g.*, 160 mg/day, mifepristone (RU 486, Mifeprex®, ~~*e.g.* 200mg/day~~ *e.g.*, 200 mg/day); and other compounds with estrogen/antiestrogenic activity, (*e.g.*, phytoestrogens, flavones, isoflavones and coumestan derivatives, lignans, and industrial compounds with phenolic ring (*e.g.*, DDT)). Also, anti-GnRH vaccines (see, *e.g.*, Hsu *et al.*, (2000) *Cancer Res.* 60:3701; Talwar, (1999) *Immunol. Rev.* 171:173-92), or any other pharmaceutical which mimics the effects produced by the aforementioned drugs, may also be used. In addition, steroid receptor based modulators, which may be targeted to be thymic specific, may also be developed and used. Many of these mechanisms of inhibiting sex ~~steroid signalling~~ steroid-signaling are well known. Each ~~drugs~~ drug may also be used in modified form, such as acetates, citrates and other salts thereof, which are well known to those in the art.

Please replace the paragraph on page 32, lines 5-10, with the following amended paragraph:

Because of the complex and interwoven feedback mechanisms of the hormonal system, administration of sex steroids may result in inhibition of sex ~~steroid-signalling~~ steroid-signaling. For example, estradiol decreases gonadotropin production and sensitivity to GnRH action. However, higher levels of estradiol result in gonadotropin surge. Likewise, progesterone influences frequency and amount of LH release. In men, testosterone inhibits gonadotropin production. Estrogen administered to men decreases LH and testosterone, and anti-estrogen increases LH.

Please replace the paragraph bridging pages 32 and 34, with the following amended paragraph:

In some embodiments, the sex ~~steroid mediated~~ steroid-mediated signaling to the thymus is disrupted by administration of a sex steroid analog, such as an analog of leutinizing hormone-releasing hormone (LHRH). ~~Sex steroid analogs and their use in therapies and chemical castration are well known.~~ Sex steroid analogs are commercially known and their use in therapies and chemical castration are well known. Such analogs include, but are not limited to, the following agonists of the LHRH receptor (LHRH-R): buserelin (*e.g.*, buserelin acetate, trade names Suprefact® (*e.g.*, 0.5-02 mg s.c./day), Suprefact Depot®, and Suprefact® Nasal Spray (*e.g.*, 2 μg per nostril, every 8 hrs.), Hoechst, also described in U.S. Patent Nos. 4,003,884, 4,118,483, and 4,275,001); Cystorelin® (*e.g.*, gonadorelin diacetate tetrahydrate, Hoechst); deslorelin (*e.g.*, ~~desorelin~~ deslorelin acetate, Deslorell®, Balance Pharmaceuticals); gonadorelin (*e.g.*, gonadorelin hydrocholoride, trade name Factrel® (100 μg i.v. or s.c.), Ayerst Laboratories); goserelin (goserelin acetate, trade name Zoladex®, AstraZeneca, ~~Aukland~~

Auckland, NZ, also described in U.S. Patent Nos. 4,100,274 and 4,128,638; GB 9112859 and GB 9112825); histrelin (*e.g.*, ~~histerelin~~ histrelin acetate, Supprelin®, (~~s.c.,10 µg/kg.day~~ s.c., 10 µg/kg/day), Ortho, also described in EP 217659); leuprolide (leuprolide acetate, trade name Lupron® or Lupron Depot®; Abbott/TAP, Lake Forest, IL, also described in U.S. Patent Nos. ~~4,490,291~~ 4,490,291, 3,972,859, 4,008,209, 4,992,421, and 4,005,063; DE 2509783); leuprorelin (*e.g.*, ~~leuproelin~~ leuprorelin acetate, trade name Prostap SR® (*e.g.*, single 3.75 mg dose s.c. or i.m./month), Prostap3® (*e.g.*, single ~~11.25mg~~ 11.25 mg dose s.c. every 3 months), Wyeth, USA, also described in Plosker et al., (1994) Drugs 48:930); lutrelin (Wyeth, USA, also described in U.S. Patent No. 4,089,946); Meterelin® (*e.g.*, Avorelina (*e.g.*, 10-15 mg slow-release formulation), also described in ~~EP 23904 and~~ WO 91/18016); nafarelin (*e.g.*, trade name Synarel® (i.n. 200-1800 µg/day), Syntex, also described in U.S. Patent No. 4,234,571; ~~W0 93/15722~~ WO 93/15722; and ~~EP 52510~~ EP0052510); and triptorelin (*e.g.*, triptorelin pamoate; trade names Trelstar LA® (11.25 mg over 3 months), Trelstar LA Debioclip® (pre-filled, single dose delivery), LA Trelstar Depot® (3.75 mg over one month), and Decapeptyl®, Debiopharm S.A., ~~Switserland~~ Switzerland, also described in U.S. Patent Nos. 4,010,125, 4,018,726, 4,024,121, and 5,258,492; EP 364819). LHRH analogs also include, but are not limited to, the following antagonists of the LHRH-R: abarelix (trade name Plenaxis™ (*e.g.*, 100 mg i.m. on days 1, 15 and 29, then every 4 weeks thereafter), Praecis Pharmaceuticals, Inc., Cambridge, MA) and cetrorelix (*e.g.*, cetrorelix acetate, trade name Cetrotide™ (*e.g.*, 0.25 or 3 mg s.c.), Zentaris, Frankfurt, Germany). Additional sex steroid analogs include Eulexin® (*e.g.*, flutamide (*e.g.*, 2 capsules 2x/day, total 750 mg/day), Schering-Plough Corp., also described in FR 7923545, WO 86/01105 and PT 100899), and dioxane derivatives ~~(e.g., those described in EP 413209)~~, and other LHRH ~~analogues~~ analogs such as are described in EP 181236, U.S. Patent Nos. 4,608,251, 4,656,247, 4,642,332, 4,010,149, 3,992,365, and 4,010,149. Combinations of agonists,

combinations of antagonists, and combinations of agonists and antagonists are also included. One non-limiting analog of the invention is deslorelin (described in U.S. Patent No. 4,218,439). For a more extensive ~~list, of~~ list of analogs, see Vickery et al. (1984) LHRH and Its Analogs: Contraceptive & Therapeutic Applications (Vickery et al., eds.) MTP Press Ltd., Lancaster, PA. Each analog may also be used in modified form, such as acetates, citrates and other salts thereof, which are well known to those in the art.

Please replace the paragraph at page 37, lines 13-27, with the following amended paragraph:

The intracellular receptors are members of the nuclear receptor superfamily. They are located in the cytoplasm of the cell and are transported to the nucleus after binding with the sex steroid hormone where they alter the transcription of specific genes. Receptors for the sex steroid hormones exist in several forms. Well known in the literature are two forms of the progesterone receptor, PRA and PRB, and three forms of the estrogen receptor, ERα, ERβ1 and ERβ2. Transcription of genes in response to the binding of the sex steroid hormone receptor to the steroid response element in the promoter region of the gene can be modified in a number of ways. Co-activators and co-repressors exist within the nucleus of the target cell that can modify binding of the steroid-receptor complex to the DNA and thereby effect transcription. The identity of many of these co-activators and co-repressors are known and methods of modifying their actions on steroid receptors are the topic of current research. Examples of the transcription factors involved in sex steroid hormone action are NF-1, SP1, ~~Oct-1and~~ Oct-1 and TFIID. These co-regulators are required for the full action of the steroids. Methods of modifying the actions of these nuclear regulators could involve the balance

between activator and repressor by the use of antagonists or through control of expression of the genes encoding the regulators.

Please replace the paragraph at page 40, lines 13-18, with the following amended paragraph:

As will be understood by persons skilled in the art at least some of the means for disrupting sex steroid signalling to the thymus will only be effective as long as the appropriate compound is administered. As a result, an advantage of certain embodiments of the present invention is that once the desired immunological affects of the present invention have been achieved, (2-3 months) the treatment can be stopped and ~~thee~~ the ~~subjects~~ subject's reproductive system will return to normal.

Please replace the paragraph at page 43, lines 7-11, with the following amended paragraph:

_____Those skilled in the art would be able to develop suitable anti-HIV constructs for use in the present invention. Indeed, a number of anti-HIV antisense constructs and ribozymes have already been developed and are described, for example; in U.S. Patent No. 5,811,275, U.S. Patent No. 5,741,706, PCT Publication No. WO 94/26877, Australian Patent Application No. 56394/94 and U.S. Patent No. 5,144,019.

Please replace the paragraph bridging pages 52 and 53, with the following amended paragraph:

Moreover, the ability to enhance the uptake into the thymus of hematopoietic stem cells means that the nature and type of dendritic cells can be manipulated. For example, the stem cells could be transfected with specific gene(s) which eventually become expressed in the dendritic cells in the thymus (and elsewhere in the body). In one non-limiting example of the invention, where the donor is related to the recipient but expresses an additional MHC molecule or a molecule expressed by the Y chromosome (*e.g.*, where the recipient is female and the donor is male), the genes encoding that molecule could be transfected and expressed in either the donor's HSC before reconstitution of the recipient with the donor's HSC, or could <u>be</u> transfected and expressed in the recipient's own HSC (*e.g.*, collected from the recipient prior to or concurrent with sex steroid ablation). Some of the HSC, whether donor or recipient, would then develop into dendritic cells, and so educate the newly formed T cells that the additional molecule is "self". T cells thus educated, when encountering such a molecule expressed by the donor graft tissue, will recognize the tissue as self and not attempt to reject it. Indeed, positive selection can involve multiple cell types: the cortical epithelium provides the specific differentiation molecules and third party cells provide the MHC/peptide ligands.

Please replace the paragraph at page 53, lines 13-18, with the following amended paragraph:

**Animals.** CBA/CAH and C57Bl6/J male mice were obtained from Central Animal Services, Monash University and were housed under conventional conditions. C57Bl6/J Ly5.1$^+$ were obtained from the ~~Central Animal Services Monash University~~

Central Animal Services, Monash University, the ~~Walterand~~ Walter and Eliza Hall Institute for Medical Research (Parkville, Victoria) and the A.R.C. (Perth, Western Australia) and were housed under conventional conditions. Ages ranged from 4-6 weeks to 26 months of age and are indicated where relevant.

Please replace the paragraph at page 58, lines 17-24, with the following amended paragraph:

The DN subpopulation, in addition to the thymocyte precursors, contains ~~(αβTCR +CD4-CD8-~~ $\alpha\beta TCR^{+}CD4^{-}CD8^{-}$ thymocytes, which are thought to have downregulated both co-receptors at the transition to SP cells (Godfrey & Zlotnik, 1993). By gating on these mature cells, it was possible to analyze the true TN compartment ($CD3^{-}CD4^{-}CD8^{-}$) and their subpopulations expressing CD44 and CD25. Figures 5H, 5I, 5J, and 5K illustrate the extent of proliferation within each subset of TN cells in young, old and castrated mice. This showed a significant ($p<0.001$) decrease in proliferation of the TN1 subset ($CD44^{+}CD25^{-}$ $CD3^{-}CD4^{-}CD8^{-}$), from ~~10%%~~ 10% in the normal young to around 2% at 18 months of age ~~(Fig. 5H)~~ (Fig. 5H) which was restored by 1 week post-castration.

Please replace the paragraph at page 59, lines 5-20, with the following amended paragraph:

Anti-keratin staining (pan-epithelium) of 2 year old mouse thymus, revealed a loss of general thymus architecture with a severe epithelial cell disorganization and absence of a distinct cortico-medullary junction. Further analysis using the MAbs, MTS 10 (medulla) and MTS44 (cortex), showed a distinct reduction in cortex size with age, with a less substantial decrease in medullary epithelium (data not shown). Epithelial

cell free regions, or keratin negative areas (KNA's, van Ewijk *et al.*, 1980; Godfrey *et al.*, 1990; Bruijntjes *et al.*, 1993) were more apparent and increased in size in the aged thymus, as evident with anti-cytokeratin labeling. There was also the appearance of thymic epithelial "cyst-like" structures in the aged thymus particularly noticeable in medullary regions (data not shown). Adipose deposition, severe decrease in thymic size and the decline in integrity of the cortico-medullary junction were shown conclusively with the anti-cytokeratin staining (data not shown). The thymus began to regenerate by 2 weeks post-castration. This was evident in the size of the thymic lobes, the increase in cortical epithelium as revealed by MTS 44, and the localization of medullary epithelium. The medullary epithelium ~~is~~ was detected by MTS 10 and at 2 weeks, there are still subpockets of epithelium stained by MTS 10 scattered throughout the cortex. By 4 weeks post-castration, there was a distinct medulla and cortex and discernible cortico-medullary junction (data not shown).

Please replace the paragraph at page 60, lines 5-10, with the following amended paragraph:

The thymic extracellular matrix, containing important structural and cellular adhesion molecules such as collagen, laminin and fibrinogen, was detected by the mAb MTS 16. Scattered throughout the normal young thymus, the nature of MTS 16 expression became more widespread and interconnected in the aged thymus. Expression of MTS 16 was increased further at 2 weeks post-castration while at 4 weeks post-castration, this expression was representative of the situation in the 2 month thymus (data not shown).

Please replace the paragraph at page 67, lines 11-18, with the following amended paragraph:

The above findings indicate a defect in the thymic epithelium ~~rendering it~~ rendering it incapable of providing the developing thymocytes with the necessary stimulus ~~for, development~~ for development. However, the symbiotic nature of the ~~thymic, epithelium~~ thymic epithelium and thymocytes makes it difficult to ascertain the exact pathway of destruction by the sex steroid influences. The medullary epithelium requires cortical T cells for its proper development and maintenance. Thus, if this population is ~~diminished, .the~~ diminished, the medullary thymocytes may not receive adequate signals for development. This particularly seems to affect the $CD8^+$ population. $IRF^{-/-}$ mice show a decreased number of $CD8^+$ T cells. It would therefore, be interesting to determine the proliferative capacity of these cells.

Please replace the paragraph at page 73, lines 13-23, with the following amended paragraph:

In both irradiation and cyclophosphamide models of immunodepletion thymocyte numbers peaked at ~~every~~ two weeks and decreased four weeks after treatment. Almost immediately after irradiation or chemotherapy, thymus weight and cellularity decreased dramatically and approximately 5 days later the first phase of thymic regeneration begun. The first wave of reconstitution (days 5-14) was brought about by the proliferation of radioresistant thymocytes (predominantly double negatives) which gave rise to all thymocyte subsets (Penit and Ezine 1989). The second decrease, observed between days 16 and 22 was due to the limited proliferative ability of the radioresistant cells coupled with a decreased production of thymic precursors by the bone marrow (also effected by irradiation). The second regenerative phase was due

to the replenishment of the thymus with bone marrow derived precursors (Huiskamp *et al.*, 1983).

Please replace the paragraph at page 77, lines 3-16, with the following amended paragraph:

In noncastrated mice, there was a profound decrease in thymocyte number over the 4 week time period, with little or no evidence of regeneration (Fig. 21A). In the castrated group, however, by two weeks there was already extensive thymopoiesis which by four weeks had returned to control levels, being 10 fold higher than in noncastrated mice. Flow cytometeric analysis of the thymii with respect to CD45.2 (donor-derived antigen) demonstrated that no ~~donor-derived~~ donor-derived cells were detectable in the noncastrated group at 4 weeks, but remarkably, virtually all the thymocytes in the castrated mice were donor-derived at this time point (Fig. 21B). Given this extensive enhancement of thymopoiesis from donor-derived haemopoietic precursors, it was important to determine whether T cell differentiation had proceeded normally. CD4, CD8 and TCR defined subsets were ~~analysed~~ analyzed by flow cytometry. There were no proportional differences in thymocytes subset proportions 2 weeks after reconstitution (Fig. 22). This observation was not possible at 4 weeks, because the noncastrated mice were not reconstituted with donor-derived cells. However, at this time point the thymocyte proportions in castrated mice appear normal.

Please replace the paragraph at page 82, lines 19-26, with the following amended paragraph:

The patient was given sex steroid ablation therapy in the form of delivery of an LHRH agonist. This was given in the form of either Leucrin (depot injection; ~~22.5mg~~ 22.5 mg) or Zoladex (implant; 10.8 mg), either one as a single dose effective for 3 months. This was effective in reducing sex steroid levels sufficiently to reactivate the thymus. In other words, the serum levels of sex steroids were undetectable (castrate; ~~<0.5ng/ml~~ <0.5 ng/ml blood). In some cases it is also necessary to deliver a suppresser of adrenal gland production of sex steroids. Caused (~~5mg/day~~ 5 mg/day) as one tablet per day may be delivered for the duration of the sex steroid ablation therapy. Adrenal gland production of sex steroids makes up around 10-15% of a human's steroids.

Please replace the paragraph bridging pages 82 and 83, with the following amended paragraph:

Reduction of sex steroids in the blood to minimal values took about 1-3 weeks; concordant with this was the reactivation of the thymus. In some cases it is necessary to extend the treatment to a second 3 month injection/implant. The thymic expansion may be increased by simultaneous enhancement of blood HSC either as an allogeneic donor (in the case of grafts of foreign tissue) or autologous HSC (by injecting the host with G-CSF to mobilize these HSC from the bone marrow to the ~~thymus~~ thymus).

Please replace the paragraph at page 84, lines 8-24, with the following amended paragraph:

Where practical, the level of hematopoietic stem cells (HSC) in the donor blood is enhanced by injecting into the donor granulocyte-colony stimulating factor (G-CSF) at ~~10μg/kg~~ 10 μg/kg for 2-5 days prior to cell collection (*e.g.*, one or two injections of ~~10μg/kg~~ 10 μg/kg per day for each of 2-5 days). $CD34^+$ donor cells are purified from the donor blood or bone marrow, such as by using a flow cytometer or immunomagnetic beading. Antibodies that specifically bind to human CD34 are commercially available (from, *e.g.*, Research Diagnostics Inc., Flanders, NJ). Donor-derived HSC are identified by flow cytometry as being $CD34^+$. These CD34+ HSC may also be expanded by in vitro culture using feeder cells (*e.g.*, fibroblasts), growth factors such as stem cell factor (SCF), and LIF to prevent differentiation into specific cell types. At approximately 3-4 weeks post LHRH agonist delivery (*i.e.*, just before or at the time the thymus begins to regenerate) the patient is injected with the donor HSC, optimally at a dose of about 2-4 x $10^6$ cells/kg. G-CSF may also be injected into the recipient to assist in expansion of the donor HSC. If this timing schedule is not possible because of the critical nature of clinical condition, the HSC could be administered at the same time as the GnRH. It may be necessary to give a second dose of HSC 2-3 weeks later to assist in the thymic regrowth and the development of donor DC (particularly in the thymus). Once the HSC have ~~engraftment~~ engrafted (*i.e.*, have incorporated into the bone marrow and thymus), the effects should be permanent since the HSC are self-renewing.